

# IMMUNOREACTIVE PROTEINS OF *BRACHYSPIRA HYODYSENTERIAE* IN PIGS

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## Introduction

Swine dysentery (SD), caused by the anaerobic spirochete *Brachyspira hyodysenteriae*, is a major problem in swine industry worldwide. Pigs suffering from SD mainly present with mucous and/or hemorrhagic diarrhea and weight loss. Few antimicrobial drugs are active against *B. hyodysenteriae* and resistance against these drugs is regularly reported<sup>1</sup>.

The introduction of asymptomatic carrier animals represents an important threat for negative herds. To date, diagnosis of SD is based on clinical signs, culture and/or PCR tests. However, no reliable diagnostic tests are commercially available to verify the SD status of a herd<sup>2</sup>. A serological test to detect the presence of SD in a herd would be a valuable tool. By use of two-dimensional western blot (2D-WB) and mass spectrometry (MS) we aimed to identify immunoreactive proteins of *B. hyodysenteriae* that could be useful for the development of diagnostic tests.

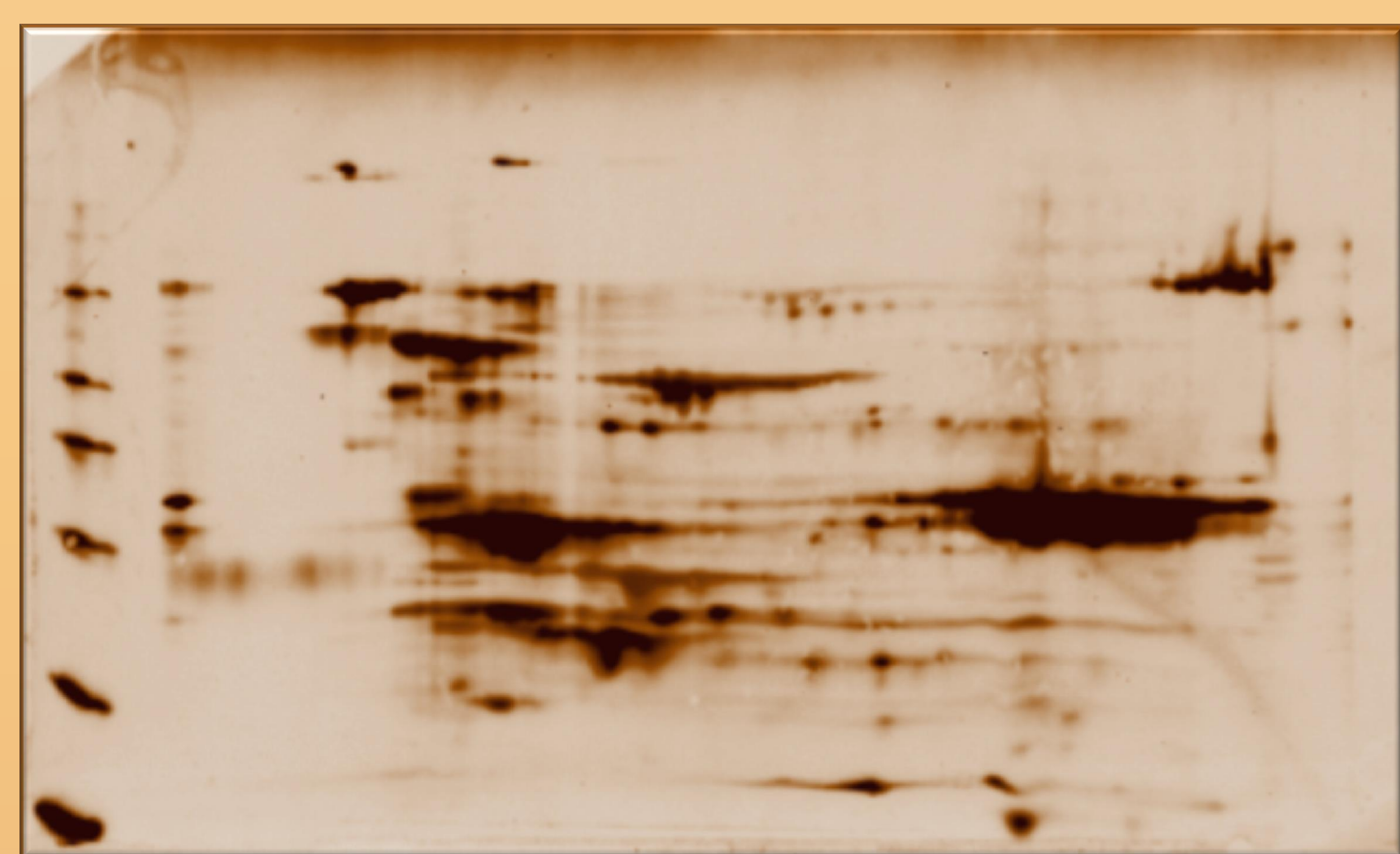
## Material and methods

Sera from pigs, experimentally infected with *B. hyodysenteriae*, that showed clinical signs of SD were collected. A total protein extract of *B. hyodysenteriae* was obtained<sup>3</sup>. Sera and total protein extract were used in a 2D-WB protocol to separate and subsequently visualize proteins of *B. hyodysenteriae* that are recognized by antibodies of infected pigs<sup>4</sup>. Spots of interest were excised from the protein gel and digested with trypsin prior to MS.

In a modified 2D-WB protocol, serum of an infected pig was incubated with a pool of total protein extracts of *B. innocens*, *B. intermedia*, *B. pilosicoli*, *B. murdochii* and “*B. hampsonii*” to capture antibodies that also reacted with proteins from these *Brachyspira* species, thus selecting for antigens specific to *B. hyodysenteriae*.

## Results

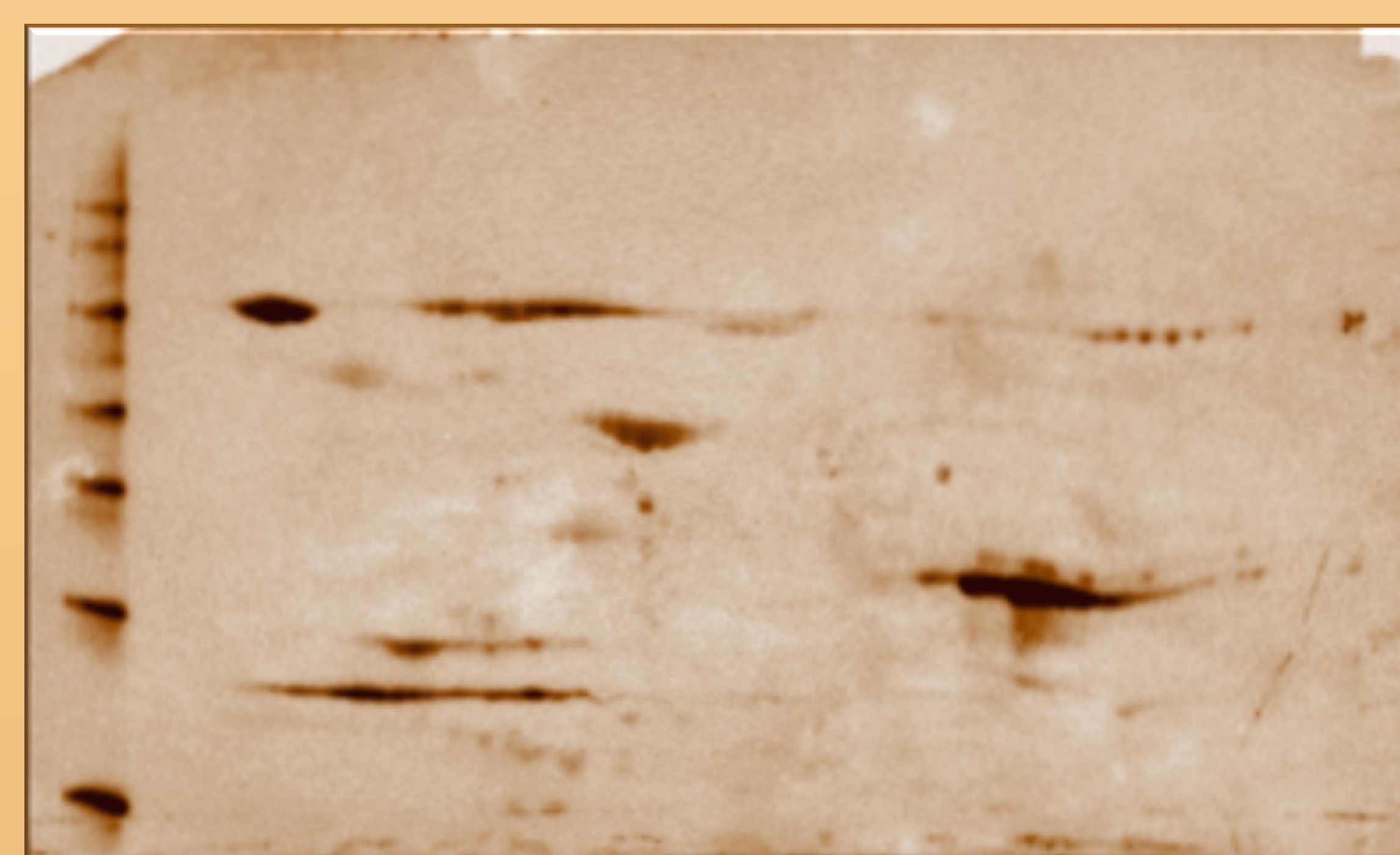
2D-WB and MS with sera from experimentally infected pigs resulted in a comprehensive list of potential immunoreactive proteins of *B. hyodysenteriae*. Total protein extracts of other *Brachyspira* species showed important similarities to that of *B. hyodysenteriae*. Preabsorption of the primary serum with total protein extracts of these *Brachyspira* species offered a solution to this problem and resulted in a less extensive pattern on the blots compared to the regular 2D-WB protocol.



Original



Preabsorbed  
serum



## Discussion

Asymptomatic carrier animals are an important risk factor for the spread of SD between herds. A serological test could detect these dormant sources of SD and facilitate diagnosis and prevention.

A drawback in the search of proteins unique for *B. hyodysenteriae* and potentially useful for diagnostics is the similarity between *Brachyspira* species. Using our adapted 2D-WB protocol followed by MS we were able to compensate for this problem and identify proteins of *B. hyodysenteriae* that are possible candidates for the development of diagnostic tests and vaccines. Further research is needed to confirm the importance of these proteins of *B. hyodysenteriae* by recombinant expression followed by one-dimensional gel electrophoresis.

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